REMARKS

After entry of this amendment, claims 7-11 are pending. Claims 7, 10 and 11 have been amended without prejudice or disclaimer to delete the non-elected subject matter. Applicant reserves all rights to pursue the non-elected subject matter in one or more divisional applications. Claim 7 as amended finds support *inter alia* in the specification at page 4, line 1 and page 27, lines 7-9. Claim 10 as amended finds support in the specification at page 1, line 14. No new matter has been added.

Priority

The Examiner acknowledged all of the priority claims made in this application. The Examiner stated, however, that the effective priority date of the present application is March 26, 2004 because the Examiner was not able to find support for the present invention in the priority documents EPO 03019642.2, PCT/EP2003/013980, EPO 04001895.4, and EPO 04001894.7. Applicant respectfully disagrees.

As the Examiner correctly pointed out, the present invention is directed to a method for identifying a gamma secretase modulator comprising identifying a FADS2-interacting molecule. Support for the claimed invention is found at least in the earliest priority document, EPO 03019642.2 filed on September 5, 2003. As stated in the specification of EPO 03019642.2, at page 292, No. 26, a method for identifying modulators of the complex No. 8 is disclosed. Complex No. 8 is described as a complex involved in the gamma secretase activity. See page 284, No. 8. Furthermore, the specification of EPO 03019642.2 discloses such modulators can be identified by screening a molecule that binds to Δ6 fatty acid desaturase and determining the binding of such a molecule. See page 293-294, No. 31, item (x). Δ6 fatty acid desaturase is also known as FADS2. See Specification at page 4, line 1. Additionally, the 100% identity of the sequence of Δ6 fatty acid desaturase as disclosed in EPO 03019642.2 (i.e. SEQ ID NO: 166) and the sequence of FADS2 as disclosed in the present application (i.e. SEQ ID NO: 76) indicates that they correspond to the same protein. Moreover, the specification of EPO 03019642.2 further discloses a method for preparing a pharmaceutical composition for the treatment of neurodegenerative diseases as claimed in claim 11. See page 298, Nos. 32 and 33. Thus, it is respectfully submitted that the subject matter of claims 7-11 is supported by the disclosure of the priority document EPO 03019642.2, and thus, the effective priority date of the present

application should be the filing date of EPO 03019642.2, September 5, 2003. Reconsideration is respectfully requested.

Claim Objection

Claims 7-11 were objected to for the use of improper abbreviations. In view of the present amendment, the objection is believed to be moot. Reconsideration and withdrawal of this objection is respectfully requested.

Rejection under 35 U.S.C. § 112, second paragraph

Claims 7-11 were rejected under 35 U.S.C. § 112, second paragraph, for allegedly being incomplete for omitting essential steps. In response, claim 7 has been amended to specify that a given test compound is identified as a FADS2-interacting molecule via its binding to FADS2. Methods useful for determining whether a given test compound binds to FADS2 are disclosed in the specification at pages 28-30 in detail. Thus, Applicant respectfully requests reconsideration and withdrawal of this rejection.

Rejection under 35 U.S.C. § 103

Claims 7-11 were rejected under 35 U.S.C. § 103(a) as being obvious over Winther et al. and Fechteler et al. Applicant respectfully disagrees and traverses the rejection.

Winther et al. teach identification of molecules that modulate FADS2 activity and their use for the treatment of Alzheimer's syndrome. See Winther et al. at page 7, 3rd full paragraph, page 8, 3rd full paragraph, and the paragraph bridging pages 8 and 9. Winther et al., however, do not teach or suggest that the identified molecules could modulate gamma secretase activity, or that gamma secretase modulation of such molecules should be determined.

Fechteler et al. disclose identification of membrane-bound protease inhibitors, particularly gamma secretase inhibitors. Fechteler et al. do not teach or suggest that these gamma secretase inhibitors could be FADS2 interacting molecules.

At the time the present application was filed, it was known in the art that molecules beneficial in treating Alzheimer's disease might have completely diverse mode of action and involve various pathways or mechanisms in the cell. Examples include cholinesterase inhibitors that increase availability of acetylcholine, or NMDA receptor antagonists that protect against

glutamate excitotoxicity. As summarized in a review article authored by Dr. P. M. Doraiswamy (CNS Drugs, 2002, 16(12): 811-824), different approaches involving different mode of action may be used for the treatment of Alzheimer's disease. See Doraiswamy at page 820, Table I, a copy of which is attached for the Examiner's reference. Note that none of the approaches mentioned in the Doraiswamy article involves modulation of enzymes involved in fatty acid metabolism, as taught in the present application. Absent the hindsight afforded by a reading of Applicant's disclosure, a person of ordinary skill, upon reading Winther et al. and Fechteler et al., would not have motivation to test whether the molecules identified in Winther et al. might also modulate gamma secretase activity. Thus, it is respectfully submitted that the disclosure of Fechteler et al. does not remedy the lack of teaching of Winther et al. Accordingly, the subject matter as claimed would not have been obvious in view of the cited references, alone or in combination.

Reconsideration and withdrawal of the obviousness rejection is respectfully requested.

CONCLUSION

For at least the above reasons, Applicant respectfully requests withdrawal of the rejections and allowance of the claims.

Applicant reserves all rights to pursue the non-elected subject matter in one or more divisional applications.

Accompanying this response is a petition for a three-month extension of time to and including July 9, 2007, to respond to the Office Action mailed January 9, 2007 with the required fee authorization. No further fees are believed due. However, if any additional fee is due, please charge our Deposit Account No. 03-2775, under Order No. 14129-00001-US from which the undersigned is authorized to draw.

Respectfully submitted,

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Attachment: P. M. Doraiswamy, Non-Cholinergic Strategies for Treating and Preventing Alzheimer's Disease, CNS Drugs, 2002, 16(12): 811-824.

REVIEW ARTICLE

CNS Drugs 2002; 16 (12): 811-824
1172-7047/02/0012-0811/$25.00/0

Non-Cholinergic Strategies for Treating and Preventing Alzheimer's Disease

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Contents

Abstract

The pathophysiology of Alzheimer's disease is complex and involves several different biochemical pathways. These include defective β-amyloid (Aβ) protein metabolism, abnormalities of glutamatergic, adrenergic, serotonergic and dopaminergic neurotransmission, and the potential involvement of inflammatory, oxidative and hormonal pathways. Consequently, these pathways are all potential targets for Alzheimer's disease treatment and prevention strategies. Currently, the mainstay treatments for Alzheimer's disease are the cholinesterase inhibitors, which increase the availability of acetylcholine at cholinergic synapses. Since the cholinesterase inhibitors confer only modest benefits, additional non-cholinergic Alzheimer's disease therapies are urgently needed.

Several non-cholinergic agents are currently under development for the treatment and/or prevention of Alzheimer's disease. These include anti-amyloid strategies (e.g. immunisation, aggregation inhibitors, secretase inhibitors), transition metal chelators (e.g. clioquinol), growth factors, hormones (e.g. estradiol), herbs (e.g. *Ginkgo biloba*), nonsteroidal anti-inflammatory drugs (NSAIDs, e.g. indomethacin), antioxidants, lipid-lowering agents, antihypertensives, selective phosphodiesterase inhibitors, vitamins (E, B12, B6, folic acid) and agents that target neurotransmitter or neuropeptide alterations. Neurotransmitter receptor–based

approaches include agents that modulate certain receptors (e.g. nicotinic, muscarinic, α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid [AMPA], γ-aminobutyric acid [GABA], *N*-methyl-D-aspartate [NMDA]) and agents that increase the availability of neurotransmitters (e.g. noradrenergic reuptake inhibitors).

Of these strategies, the NMDA receptor antagonist memantine is in the most advanced stage of development in the US and is already approved in Europe as the first treatment for moderately severe to severe Alzheimer's disease. Memantine is proposed to counteract cellular damage due to pathological activation of NMDA receptors by glutamate. Results with *Ginkgo biloba* have been mixed. Data for neurotrophic therapies and vitamin E (tocopherol) appear promising but require confirmation. NSAIDs and conjugated estrogens have not proven to be of value to date for the treatment of Alzheimer's disease. Statins may have a potential role in reducing the risk or delaying the onset of Alzheimer's disease, although this has yet to be confirmed in randomised trials. There are currently no data to support the use of statins as a treatment for dementia.

This article provides an update on the current status of selected agents, focusing primarily on those agents with the most extensive clinical evidence at present.

Alzheimer's disease is the most common age-related neurodegenerative disorder, affecting an estimated 4 million people in the US alone.[1] Hallmarks of the illness include amyloid plaques and neurofibrillary tangles in the brain, as well as impairment of central cholinergic neurotransmission. Clinical manifestations of Alzheimer's disease involve not only memory loss and cognitive dysfunction, but also progressive functional deficits and various behavioural and psychological symptoms including depression, psychosis, aggression, agitation and sleep disturbance.

Until recently, the main drugs available for the specific treatment of Alzheimer's disease were cholinesterase inhibitors, which increase the availability of acetylcholine at cholinergic synapses. Drugs of this class have been widely used and are moderately effective in ameliorating the symptoms of mild to moderate Alzheimer's disease.[2,3] The most commonly used cholinesterase inhibitors, donepezil, galantamine and rivastigmine, have been studied in clinical trials of 6 to 12 months' duration and have been shown to improve both cognition and clinical impressions of global change relative to placebo in patients with mild to moderate symptoms of Alzheimer's disease.[4-6] Emerging data indicate that cholinesterase inhibitors may also slow the loss of certain activities of daily living and postpone or attenuate the emergence of certain dementia-related behaviours.[7-9] Recently, the efficacy of donepezil was evaluated in patients with moderate to severe Alzheimer's disease, and the drug was found to be superior to placebo according to measures of cognition, function and behaviour.[10] These data suggest that the benefits of donepezil may extend to more advanced stages of Alzheimer's disease, and a confirmatory study is underway.

Cholinesterase inhibitors, however, have not been shown to prevent disease progression, and few other data from well controlled studies are available to date to support their use in advanced or severe stages of Alzheimer's disease.[10] Moreover, management of many of the noncognitive psychiatric symptoms of Alzheimer's disease often requires adjunctive treatment with antidepressants, antipsychotics, mood stabilisers and/or hypnotics. Given these limitations in the available treatments for patients with Alzheimer's disease, there is a clear need for additional therapeutics.

Though pharmacological approaches have focused primarily on the augmentation of cholinergic transmission, the pathophysiology of Alzheimer's disease is complex, and altered or defective β-amyloid (Aβ) protein metabolism is increasingly implicated as the primary suspect. There is also

ample evidence that abnormalities of other CNS neurotransmitter pathways, including glutamatergic, adrenergic, serotonergic, peptidergic and dopaminergic, are likely to play a role in memory dysfunction or Alzheimer's disease.[11-13] Additional pathological mechanisms may include defective tau-metabolism, vascular changes, apoptosis, second messenger alterations, inflammation, oxidative stress and hormonal effects. The pharmacological manipulation of these non-cholinergic pathways may provide new therapeutic strategies that can be implemented over the entire course of Alzheimer's disease.

This article will review and assess selected non-cholinergic approaches to the management of Alzheimer's dementia, focusing on recent clinical findings. In addition, several neuroprotective and preventative strategies for Alzheimer's disease will be discussed.

1. Non-Cholinergic Strategies for the Treatment and Prevention of Alzheimer's Disease

1.1 Memantine

Glutamate is a major excitatory neurotransmitter in the brain, and an estimated 70% of all excitatory synapses in the brain are stimulated by glutamate.[14,15] Its effects are mediated by different receptor types, one of which is the *N*-methyl-D-aspartate (NMDA) receptor, which is found at high density in the cortex and hippocampus (figure 1).

Physiologically, NMDA receptors participate in neuronal plasticity, which plays a crucial role in such processes as learning and memory formation. Pathological activation of NMDA receptors by glutamate has been identified as a potential cause of chronic neurodegeneration in a variety of dementias, including Alzheimer's disease.[16] A role for pathological NMDA receptor activation in Alzheimer's disease is supported by *in vitro* studies that show that Aβ, which accumulates to form amyloid plaques in patients with the disease, increases the release of glutamate upon neuronal depolarisation.[17] This effect may be mediated by Aβ-induced stimulation of nitric oxide production by microglia,[18] since nitric oxide enhances glutamate release.[19] Aβ also inhibits glial uptake of glutamate,[20] and glutamate uptake is significantly lower in astrocytes derived from patients with Alzheimer's disease relative to cells from control individuals without dementia.[21] Excitotoxic brain injury, in turn, has been reported to result in increased amyloid precursor protein levels, introducing the possibility of a feed-forward loop.[22]

Several preclinical studies have shown that NMDA receptor antagonists prevent glutamate-induced neurotoxicity.[23-27] However, the high affinity NMDA receptor blockers phencyclidine (PCP) and dizocilpine also confer serious adverse effects, including psychosis.[28] Such adverse effects are thought to arise from the inhibition of both pathological and physiological NMDA receptor

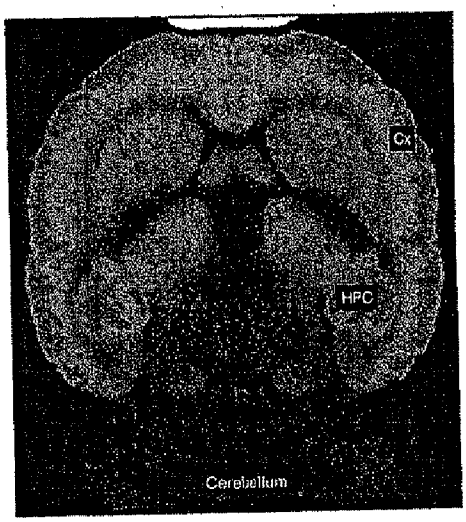

Fig. 1. Autoradiographic image of a horizontal section of a rat brain showing the distribution of glutamate *N*-methyl-D-aspartate (NMDA) receptors. The density of NMDA receptors is indicated by a colour scale (red = high density, purple = low density). Note the high density of NMDA receptors in the cortex (Cx) and also in the hippocampus (HPC), which plays a crucial role in learning and memory (figure kindly provided by Dr Anat Biegon, Lawrence Berkeley National Laboratories, Berkeley, CA, USA).

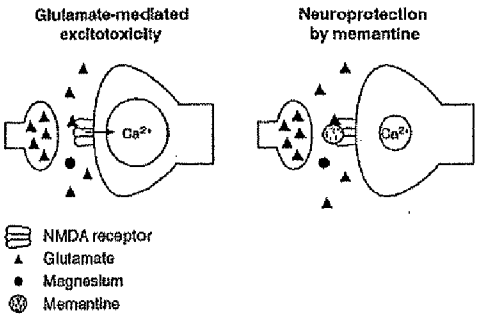

Fig. 2. Schematic of glutamate-mediated excitotoxicity via overactivation of *N*-methyl-D-aspartate (NMDA) receptors and neuroprotection by memantine binding to these receptors (reproduced from Danysz et al.,[34] with permission from Taylor & Francis Ltd., http://www.tandf.co.uk/journals). Glutamate excitotoxicity (left) is thought to play a role in several neurodegenerative disorders, including Alzheimer's disease.[16] Memantine, an NMDA receptor antagonist, blocks excitotoxic glutamate activity (right). Because of its moderate affinity and voltage sensitivity, memantine does not appear to interfere with physiological processes governed by activation of NMDA receptors.[29]

activity.[29] In contrast, some moderate- to low-affinity receptor antagonists appear to selectively inhibit the pathological activation of the receptor.[29] Therefore, recent research has focused on the use of moderate-affinity agents that block the NMDA receptor ion channel.

The most widely studied of these moderate-affinity antagonists *in vivo* is memantine.[29] Memantine has been reported to exhibit neuroprotective effects (see figure 2 for an outline of the mechanism of this effect) in many experimental paradigms both *in vivo* and *in vitro*.[14,30-33] For example, memantine protected cultured rat cortical neurons from glutamate (100 μM)-induced toxicity, with the concentration that produced 50% of the maximal effect (IC_{50}) of 1.4 μmol/L.[31] In primary cultures of rat hippocampal neurons exposed to 1 mmol/L glutamate, memantine exhibited dose-dependent neuroprotection (IC_{50} = 1.1 μmol/L).[14] In rats injected with Aβ, therapeutically relevant doses of memantine were shown to reduce Aβ-induced neurotoxicity and apoptosis in the hippocampus and improve learning.[32] Memantine (10 to 20 mg/kg, given intraperitoneally 60 minutes prior to the induction of ischaemia) protected against focal ischaemia in rats and mice caused by middle cerebral artery occlusion and also protected hippocampal neurons from global ischaemia.[30]

Recently, another such study examined the protective effect of memantine against neurotoxicity induced in the rat cholinergic nucleus basalis of Meynert (NBM), a brain structure known to be affected in Alzheimer's disease.[33] Infusion of the NMDA receptor agonist and toxin quinolinic acid directly into the rat NBM causes effects that mimic some of the symptoms of Alzheimer's disease: a decrease in cholinergic nerve terminals, as measured by choline acetyl transferase (ChAT) activity, and learning defects. Pre-treatment with a therapeutically relevant dose of memantine attenuated the decline in ChAT activity and the learning defects induced by quinolic acid.

Memantine has been used in Germany for over a decade to treat dementia, and it has recently been approved in Europe by the European Union Committee for Proprietary Medicinal Products (CPMP) for the treatment of moderately severe to severe Alzheimer's disease. A recent clinical trial conducted in the US evaluated the efficacy and safety of memantine for the treatment of patients with moderate to severe Alzheimer's disease.[35] In this double-blind, placebo-controlled, 28-week trial, memantine reportedly improved cognition and function significantly relative to placebo. Memantine was safe and well tolerated, and there were no clinically important differences noted in the number of patients who experienced adverse effects between treatment groups. In addition, in this study, memantine was associated with a significantly lower rate of patient institutionalisation and a significant reduction in caregiver time-burden relative to placebo.[36] The results of this trial are pending peer review and publication.

In an open-label extension of this same study, patients who switched from placebo to memantine showed an improvement in cognition, function and behaviour, relative to their projected rate of de-

cline.[37] These data must be interpreted with caution given the limitations of an open-label trial.

Overall, the wealth of clinical exposure outside the US, as well as the trials conducted to date both in Europe and in the US, suggest that memantine may fill an important treatment gap for patients with moderate to severe Alzheimer's disease. Currently, it is an investigational agent in the US, and studies are ongoing to assess whether this compound is beneficial in mild to moderate Alzheimer's disease in patients with or without concomitant cholinesterase inhibitor treatment.

1.2 Nonsteroidal Anti-Inflammatory Drugs

Inflammation can cause cellular damage and has been implicated as a potential pathophysiological mechanism of Alzheimer's disease based on post-mortem pathology and epidemiological studies. Hallmarks of inflammation such as activated microglial cells and proinflammatory cytokines have been found in post-mortem brain tissue from patients with Alzheimer's disease.[38] Likewise, some clinical studies have found elevated levels of inflammatory markers in the plasma or CSF of patients with the disease.[39]

Epidemiological studies have suggested an association between nonsteroidal anti-inflammatory drug (NSAID) use and improved cognition in patients with Alzheimer's disease or reduced occurrence of the disease, suggesting that NSAIDs may postpone or prevent the onset and progression of Alzheimer's disease.[40,41] However, results from prospective clinical trials have been mostly negative. A pilot trial with indomethacin reported improved cognition relative to placebo in patients with mild to moderately severe Alzheimer's disease,[42] whereas more recently the same class of patients treated with diclofenac were not statistically different from placebo-treated patients on cognitive or functional measures.[43] A large, multinational, randomised clinical trial evaluating celecoxib, a member of the relatively new NSAID class, the selective inhibitors of cyclo-oxygenase 2 (COX-2), failed to show significant efficacy for the treatment of mild to moderate Alzheimer's disease, although these data have not yet been published.[44] Finally, recent trials examining the efficacy of naproxen and rofecoxib (another selective COX-2 inhibitor) also failed to demonstrate significant benefit from either NSAID in patients with mild to moderate Alzheimer's disease.[45,46]

Recently, the focus of clinical investigation has shifted to the possible efficacy of NSAIDs for the prevention of Alzheimer's disease. However, similar to the efficacy data for the use of these drugs to treat symptoms of Alzheimer's disease, the data for prevention are mixed, though relatively more studies report a beneficial association. For example, a recent prospective, observational, population-based cohort study in the Netherlands was conducted to determine whether the use of NSAIDs other than aspirin was associated with a decreased risk of Alzheimer's disease or vascular dementia.[47] This study revealed a consistently decreasing risk of Alzheimer's disease with increasing cumulative duration of NSAID use over an 8-year period, supporting the theory that NSAIDs confer protection against Alzheimer's disease. Interestingly, a recent subanalysis of this study revealed that the use of the NSAIDs ibuprofen, indomethacin and sulindac were largely responsible for the overall decreased risk observed, whereas the use of diclofenac and naproxen contributed little to the risk reduction.[48]

In contrast, a recent retrospective case-controlled analysis of patients with Alzheimer's disease and control individuals without dementia found no significant difference in the proportion of cases and control individuals who had received NSAIDs in the 3 years prior to the onset of dementia.[49] Thus, the dose and duration of use required, as well as the type of NSAID needed to achieve maximal neuroprotective effects, is not known. The question of whether once daily aspirin can be effective in preventing Alzheimer's disease also remains controversial.[47,50-52]

It is clear that additional studies are needed to determine whether NSAIDs may have a role in reducing the risk of Alzheimer's disease, and randomised clinical trials are currently underway.

One ongoing study is a dementia primary prevention trial with naproxen and celecoxib, and another is a secondary prevention trial with ibuprofen in people with mild cognitive impairment.

1.3 *Ginkgo biloba*

Ginkgo biloba extracts have been used for thousands of years in traditional Chinese medicine for a variety of medicinal purposes. In the last several decades, ginkgo extracts have been widely used in Europe to treat age-related physical and cognitive disorders, including Alzheimer's disease. The anti-oxidative and anti-peroxidative properties of its flavonoid component and the anti-inflammatory activity of its terpenoid component are thought to be the basis for the purported efficacy of ginkgo in Alzheimer's disease.[53-56]

Ginkgo extract has shown significant positive effects on cognition relative to placebo in several, but not all, clinical trials of patients with mild to moderately severe dementia, including Alzheimer's disease.[57-59] Two comparisons of the effect sizes in various trials have suggested that the cognitive efficacy of ginkgo appears comparable to that of the cholinesterase inhibitors;[57,58] however, a third more recent comparison indicated that cholinesterase inhibitors provide greater benefit.[60] There are also negative trials with ginkgo as there are with some of the cholinesterase inhibitors, depending on dose, duration and study design.[59]

While the risk of bleeding with gingko may be linked to its antiplatelet effects,[57] there are few published data on the relative risk for such events given the millions of people who have used this herb. Of interest, results from another recent clinical trial showed significant cognitive improvements in healthy individuals treated with ginkgo (180 mg/day).[61] However, another recent study using a slightly lower dosage of ginkgo (120 mg/day) failed to find any evidence of cognitive efficacy in healthy elderly individuals.[62] Thus, the optimal dosage of ginkgo for use in different settings remains controversial.

A large controlled trial of ginkgo to examine its efficacy and safety in the prevention of dementia in older people is underway in the US, and its results will influence future prescribing patterns of ginkgo. There is also a need for a direct, randomised comparison of cholinesterase inhibitors versus ginkgo and versus their combination in dementia, since such a study would potentially clarify further the role of ginkgo in treating Alzheimer's disease.

1.4 FPF 1070

Nerve growth factors (NGFs) have been attractive candidates for treatment of neurodegenerative disorders such as Alzheimer's disease because of their neurotrophic activity. Indeed, human β-NGF has demonstrated reversal of cholinergic neuronal damage in rodents and prevention of cholinergic neuronal degradation in primates.[63] However, the inability of NGFs to cross the blood-brain barrier poses inherent difficulties for its clinical use, since high-risk surgical techniques for drug delivery are required including intracerebral infusion, slow-release biodegradable implants or grafting of NGF-producing cells to intracerebral sites.[64] Stem cell therapy and the use of other growth factors or neurotrophic agents such as phosphodiesterase inhibitors are still in very early stages of development.[65]

More recently, it has been demonstrated that FPF 1070, a low molecular weight peptidic preparation of porcine brain proteins, can be delivered by intravenous infusion and exhibits neurotrophic activity in cells of the peripheral nervous system and CNS.[66,67]

In a series of preliminary clinical trials with 4-week FPF 1070 infusion (30ml FPF 1070 plus saline given on 5 consecutive days of each week for reported time ranges of 20 to >30 minutes), patients with mild to moderate Alzheimer's disease showed modest improvement in clinical global assessment and cognitive scores.[68-70] A recent multicentre, placebo-controlled trial using the same FPF 1070 infusion protocol with 20-minute infusions supported the earlier demonstrations of improvements in global clinical assessment scores; however, cognition was not improved relative to placebo in this trial.[71] More interestingly, the global improvement effects of treatment continued

for 2 months after the last infusion, in agreement with earlier findings.[69,70] The significance of this finding is not known, but readers should note that the cognitive benefits of donepezil in a short-term trial were lost 6 weeks after discontinuation.[72,73]

The effectiveness of FPF 1070 for the treatment of patients with moderate to severe Alzheimer's disease or for the prevention of the disease has yet to be demonstrated. It would also be of interest to determine the effects of the preparation on hippocampal and brain volume as surrogate markers of neurotrophic activity. Future studies may shed more light on the promise and role of FPF 1070 in the treatment of Alzheimer's disease.

1.5 HMG-CoA Reductase Inhibitors

Preclinical and epidemiological studies indicate that cholesterol may play an important role in the pathophysiology of Alzheimer's disease. Studies in rodents indicate that cholesterol may regulate the production of Aβ protein,[74] while epidemiological data show that the use of HMG-CoA reductase inhibitors ('statins') is associated with a reduced risk of Alzheimer's disease.[75,76] In one of these epidemiological studies, statin treatment was associated with a lower risk of dementia (of any aetiology, including Alzheimer's disease) compared with the use of other lipid-lowering treatments or untreated hyperlipidaemia for patients over the age of 50 years.[75] In another study, the prevalence of Alzheimer's disease was 60 to 73% lower in patients taking statins compared with the total patient population of the study or with patients taking other cardiac medications.[76] Similarly, a third study revealed an association between the use of statins or other lipid-lowering agents and reduced risk of dementia, including Alzheimer's disease, in subjects under the age of 80 years.[77] Thus, the effect of statins in some of these studies seemed to be independent of their lipid-lowering actions.

The mechanism by which statins exert this apparent protective effect against Alzheimer's disease is not fully understood but may involve multiple pathways, including alteration of Aβ regulation (via cholesterol-lowering activity), as well as alteration of vascular endothelial function (via activation of endothelial nitric oxide synthase).[78,79] In a recent preclinical study, two different statins, simvastatin and lovastatin, were used to treat rat hippocampal and mixed cortical neurons that expressed human Aβ.[78] Both statins strongly reduced intracellular and extracellular Aβ levels in the neuronal cultures. In addition, this study reported that high doses of simvastatin administered to guinea pigs reduced Aβ levels in CSF and brain tissue. A recent clinical study addressed the effect of statins on vascular endothelial function.[79] This study showed that pretreatment with pravastatin (20 mg/day for 2 months) significantly increased cerebral blood flow velocity in one test of vascular endothelial function.

While such findings are promising, to date there are no randomised clinical trials demonstrating the efficacy of statins relative to placebo in the prevention or treatment of Alzheimer's disease. Furthermore, it is unclear whether all statins are similarly effective or tolerable in this patient population. For example, in one case report, a patient treated with simvastatin reportedly showed progressive memory loss, which ultimately was resolved by switching the patient to pravastatin.[80] Thus, further investigation into the safety and efficacy of statin treatment for the prevention of Alzheimer's disease clearly is warranted, and at least two large clinical trials are underway.

While it is unlikely that statins will directly improve cognition, a large randomised trial should examine this issue in mild to moderate Alzheimer's disease and vascular dementia. Such a trial ideally would also permit concomitant therapy with cholinesterase inhibitors and other agents as they become available. A more promising study would be a primary dementia prevention trial of older adults stratified by baseline lipid levels. Such a study would compare a statin chosen for its anti-amyloid activity and be powered to show dementia prevention independent of its effects on lipid levels. The addition of a magnetic resonance imaging

outcome measure may also provide insights into the progression of brain ischaemia in such patients.

1.6 Estrogen

Alzheimer's disease is more prevalent in women than men.[1,81] Studies in rodents demonstrate a promotional effect of estrogen on various measures of brain activity, including acetylcholine levels, and reveal the presence of high-affinity estrogen binding sites on neurons in brain regions affected by Alzheimer's disease.[82,83] Taken together, this evidence suggests that estrogen withdrawal (e.g. resulting from menopause) may predispose to Alzheimer's disease. Although the specific biological mechanism for the proposed role of estrogen in Alzheimer's disease is not understood, estrogen has been reported to have more than 200 actions on nerve cells, including neurotrophic, anti-inflammatory and anti-oxidative activity.[84] In addition, one recent study showed that estrogen induced glutamate transport in astrocytes derived from patients who had Alzheimer's disease, suggesting that estrogen may also participate in the regulation of glutamatergic neurotransmission.[21]

Although initial, small-scale clinical trials and some epidemiological studies suggested that estrogen replacement may enhance cognition in postmenopausal women with Alzheimer's disease,[41,85,86] recent results from three larger, double-blind, placebo-controlled clinical trials do not support the use of short-term (12 to 16 weeks)[87,88] or long-term (1 year)[89] conjugated equine estrogen replacement therapy for the treatment of symptoms of mild to moderate Alzheimer's disease. However, other forms of estrogen (e.g. estradiol) may have greater cognitive benefits than conjugated equine estrogen, a notion supported by the study conducted by Asthana and colleagues.[86]

Data from a variety of recent epidemiological studies indicate that estrogen may reduce the risk of developing Alzheimer's disease.[90-93] However, since women who use estrogen replacement therapy at or following menopause are not necessarily representative of the general population at risk for Alzheimer's disease, there remains controversy about whether these findings are somehow biased by a cohort effect or other factors. Moreover, in a recent population-based study, the frequency of estrogen use was significantly higher in patients with Alzheimer's disease than in those without the disease, and this relationship between estrogen use and Alzheimer's disease remained significant after adjustment for a variety of socioeconomic and health factors.[93] A prospective clinical trial aimed at evaluating the ability of conjugated estrogen to delay the onset of Alzheimer's disease is currently underway to confirm the epidemiological findings.

It should be noted that recently, in a long-term trial examining the effect of hormone replacement therapy on coronary heart disease in women, the use of combined estrogen and progestin was stopped as a result of an increased incidence of heart attacks, strokes, blood clots and breast cancer.[94] However, an increased incidence of these adverse events was not observed in women taking estrogen alone, and that treatment group will continue to be followed. These safety findings should be considered when using hormone replacement therapy in any future clinical trials for the prevention of Alzheimer's disease.

1.7 Antioxidants

In post-mortem studies of patients who had had Alzheimer's disease, several markers of oxidative damage are found in the brain, including increased lipid peroxidation, increased protein and DNA oxidation, and a decline in the levels of polyunsaturated fatty acids (see review by Markesbery and Carney[95]). Cross-sectional and longitudinal studies have shown that elevated levels of plasma homocysteine, which can cause cholesterol oxidation, are associated with poor cognition or dementia.[96,97] A recent study of 1092 individuals without dementia showed that a plasma homocysteine level greater than 14 μmol/L at baseline nearly doubled the risk of developing Alzheimer's disease 8 years later.[98] Since homocysteine levels are also associated with vascular changes, it is unclear at

present how elevated homocysteine levels translate into risk of Alzheimer's disease. Regardless, these results have been taken to indicate that dietary supplementation with B vitamins or folate, which reduce plasma levels of homocysteine, may provide protection from Alzheimer's disease, and prospective clinical trials are underway.

Additional antioxidants have been investigated as possible preventatives for Alzheimer's disease, and epidemiological studies suggest that vitamin E and vitamin C may reduce the risk for the disease in the elderly.[99,100] Vitamin E slowed the progression to institutionalisation, inability to perform activities of daily living, and severe dementia in patients with moderate Alzheimer's disease, although these results remain controversial.[101] There is no trial evidence to support a value for vitamin E in other stages of Alzheimer's disease.

Prospective clinical trials in this patient population are needed to further assess the efficacy of these and other antioxidants for the prevention of Alzheimer's disease. A secondary prevention trial of patients with mild cognitive impairment evaluating the efficacy of vitamin E is underway.

1.8 Anti-Amyloid Strategies

Strategies for the prevention of Alzheimer's disease that directly target the Aβ protein are currently being explored. One such strategy is immunotherapy. In a recent study, transgenic mice that overexpress Aβ42, an isoform of Aβ that predominates in amyloid plaques, were used to test the efficacy of one immunisation strategy.[102] Untreated, these transgenic mice progressively develop many of the neuropathological symptoms of Alzheimer's disease, including amyloid plaques, neuritic dystrophy and astrogliosis. Immunisation of young, largely presymptomatic animals with synthetic human Aβ42 prevented amyloid plaque formation in seven of nine treated mice, while immunisation of older animals significantly reduced the extent and progression of Alzheimer's disease–like neuropathologies.

Based on these promising results in rodents, immunotherapy with synthetic human Aβ42 was recently tested in humans. Results of initial safety tests in 100 patients apparently indicated that the immunisation was safe since no significant adverse effects were reported.[103] However, phase II trials assessing this strategy in 375 patients were suspended in early 2002 when some patients developed clinical signs indicating inflammation in the CNS. Subsequently, additional patients reported similar symptoms, and the fate of this programme is unclear.[103] Since the patients in the trial have already been treated, it is believed that they will continue to be followed. It is not clear if or when results will be made public.

Other strategies such as secretase inhibitors and aggregation inhibitors are in early human testing and unlikely to reach the clinic for several years.

1.9 Chelation Therapy

The metal ions zinc, copper and iron are implicated in the formation of amyloid plaques based on *in vitro* experiments, rodent studies and human Alzheimer's disease pathology. These studies have found that the Aβ protein has copper and zinc binding sites, and that these ions, along with iron, are enriched in amyloid plaques. Enrichment of zinc and iron is found in the amyloid deposits of transgenic mouse models of Alzheimer's disease, while enrichment of copper and zinc has been found in amyloid plaques of patients with the disease.[104-107] In addition, a recent study demonstrated that copper/zinc chelators can solubilise amyloid plaques.[108]

As an extension of these results, the copper/zinc chelator clioquinol was tested for the ability to inhibit amyloid deposition in one transgenic mouse model of Alzheimer's disease.[109] In transgenic mice that exhibit Alzheimer's disease–like symptoms, including Aβ elevation and deposits, oral treatment with clioquinol for 9 weeks in a controlled setting resulted in a 49% decrease in brain Aβ deposition. Clioquinol also has shown initial promising results in humans. Clioquinol (20 mg/day and 80 mg/day) was tested in an open study of 20 patients with Alzheimer's disease, and a slight improvement in clinical ratings was observed after 3 weeks of treatment.[110] More recently, results of a

phase II, placebo-controlled trial of clioquinol in 32 patients with Alzheimer's disease reportedly showed that clioquinol treatment slowed the rate of cognitive decline in some patients.[111] However, this study has not been published and hence should be interpreted accordingly. These results support the further testing of this chelator as a novel therapy for the prevention of amyloid plaque formation in Alzheimer's disease.

1.10 Other Strategies

A variety of other strategies (e.g. growth factors, gene therapy, ampakines, steroid receptor antagonists, neuropeptide modulators) are being studied for their effects in Alzheimer's disease and aging. Of interest, nootropics such as piracetam analogues are in development for age-associated memory impairment, and other NMDA receptor

Table I. Pharmacological strategies for the treatment and prevention of Alzheimer's disease (for references see main text)[a]

Class of agent	Putative mechanism of action	Evidence/status
Cholinesterase inhibitors	Increase availability of acetylcholine; enhance blood flow	Currently, the only drugs approved by the US FDA for the treatment of mild to moderate Alzheimer's disease dementia. Trials are underway in other conditions
N-methyl-D-aspartate (NMDA) receptor antagonists (memantine)	Protect against glutamate excitotoxicity by antagonising NMDA receptor activity	Memantine has been used in Germany for over a decade to treat dementia; approved in Europe for treatment of Alzheimer's disease. A recent US clinical trial showed positive effects on cognition and function in patients with advanced Alzheimer's disease. Promising candidate for use as either monotherapy or in combination with other agents, pending US FDA review and approval
Ginkgo biloba	Anti-oxidative; weak anti-platelet activity; blood flow enhancer	Positive effects on cognition relative to placebo shown in some clinical trials of patients with dementia, including Alzheimer's disease. Large Alzheimer's disease prevention trial is currently underway
FPF 1070	Neurotrophic activity; crosses blood-brain barrier	Improvement in global assessment lasting beyond 4 weeks of treatment demonstrated. Larger scale clinical trials are needed
HMG-CoA reductase inhibitors ('statins')	Lipid lowering; alter β-amyloid regulation and/or vascular function	Epidemiological data show a reduced risk of Alzheimer's disease is associated with the use of statins. At least two large clinical trials are planned. Memory loss as an adverse effect has been described in case reports
Estrogen	Possibly neurotrophic; possibly enhances neurotransmission	Epidemiological studies suggest estrogen replacement may delay onset of Alzheimer's disease. Conjugated estrogen is not effective for treating Alzheimer's disease. Prospective clinical trials are underway to evaluate estrogen as a preventative therapy
Vitamins	Anti-oxidative; possibly reduce plasma homocysteine levels	Vitamin E may slow progression of moderate Alzheimer's disease. Elevated homocysteine levels linked to dementia. Prospective clinical trials are underway
Anti-amyloid strategies	Reduce accumulation of Aβ42 amyloid plaques	Phase II trials of a vaccine against the β-amyloid protein suspended as a result of safety issues. Other anti-amyloid strategies are under development
Chelation strategies	Dissolve amyloid plaques by chelating iron, zinc and copper	Pilot study of clioquinol showed a slight improvement in ratings after 3 weeks of treatment. Initial, unpublished results from a phase II clinical trial indicate that clioquinol may slow disease progression in some patients with Alzheimer's disease. Further testing is needed
Nonsteroidal anti-inflammatory drugs	Anti-inflammatory	Results from prospective studies of use as treatment in patients with Alzheimer's disease have been disappointing. Clinical trials are underway to assess role in prevention of the disease
Gene therapy; stem cell therapy	Promote neurogenesis	In early development

a Nonpharmacological therapies, such as exercise, caregiver support, behaviour modification and various other alternative therapies, are also being studied. These are beyond the scope of this review and hence are not included in this table.

antagonists are in early stages of clinical development.[112-114]

2. Conclusion

The pathophysiology of Alzheimer's disease is complex and likely to involve multiple, interconnected pathways. Data from both basic and clinical research have started to elucidate these complicated mechanisms and have provided the field with promising new therapeutic avenues or targets beyond cholinesterase inhibition for the treatment of the disease (table I). These emerging strategies may provide treatment options for Alzheimer's disease throughout the course of the disease, as well as possible agents for prevention.

The moderate-affinity NMDA receptor antagonist memantine is already available in Europe for treating Alzheimer's disease. Clinical data have demonstrated its efficacy in moderate to severe disease, and studies are underway to evaluate its potential use in mild to moderate disease. Results with *Ginkgo biloba* have been mixed, with some evidence existing for a positive effect on the cognitive symptoms of Alzheimer's disease. Rigorous confirmatory studies currently underway will further define the potential benefits of this extract. While the data for neurotrophic therapies appears promising, there is a need for larger, confirmatory trials. For antioxidant therapy, although there is one supportive trial of vitamin E in the treatment of moderate Alzheimer's disease, it will be important to confirm these findings in a second trial. NSAIDs and conjugated estrogen therapies currently do not have any proven value in the treatment of Alzheimer's disease. In addition, no positive data currently exist to indicate any value for the use of statins in the treatment of the symptoms of Alzheimer's disease, but they may be useful for preventing the development of the disease.

Although these therapeutic approaches have been presented separately in this review, their potential combined use should also be considered. Considering the complexity of Alzheimer's disease and that multiple aetiologies may contribute to the disease, combinations of therapeutic agents may result in the most efficacious strategy for its treatment. Some combination therapies have already been examined in clinical trials. For example, a *post hoc* analysis of one study found that estrogen replacement therapy enhanced the beneficial effects of the cholinesterase inhibitor tacrine.[115] Other studies are underway to determine whether other such additive benefits might be achieved.

Ultimately, the goal of research into Alzheimer's disease is disease prevention, and a number of preventative strategies are under investigation. Large-scale clinical trials are underway to examine the efficacy of statins, estrogen and antioxidants in the prevention of Alzheimer's disease. Larger, confirmatory trials of the chelating agent clioquinol, which showed modest preventative efficacy in initial clinical studies, are needed. Finally, the promising preclinical studies of the neuroprotective effects of the moderate affinity NMDA receptor antagonist memantine warrant the clinical evaluation of this agent for the prevention of Alzheimer's disease.

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